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SELF-ADMINISTRATION OF THE CANNABINOID RECEPTOR AGONIST WIN 55,212-2 IN DRUG-NAIVE MICE

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Marijuana is one of the most widely used illicit recreational drugs. However, contrary to the majority of drugs abused by humans, there is a general opinion that rewarding effects are not manifested by animals. We studied a synthetic cannabinoid agonist WIN 55,212-2 using an intravenous self-administration model in drug-naive mice. The results of this study show that WIN 55,212-2 was intravenously self-administered by mice in a concentration-dependent manner according to a bell-shaped curve. Thus, self-administration of WIN 55,212-2 significantly increased, with respect to the vehicle self-administration control group, at concentrations of 0.5 and 0.1 mg/kg per injection. However, at WIN 55,212-2 concentration of 0.5 mg/kg per injection, self-administration significantly decreased.

The results obtained show how WIN 55,212-2 is able to elicit both rewarding and aversive effects depending on the concentration used. Pretreatment of mice with the cannabinoid CB_1 receptor antagonist SR 141716A (0.25 mg/kg, i.p.) completely prevented WIN 55,212-2 (0.1 mg/kg per injection) self-administration, indicating that WIN 55,212-2 rewarding effects are specifically mediated by cannabinoid CB_1 receptors.

Although cannabis is known as one of the oldest and most widely abused drugs, it has proved difficult to demonstrate its reinforcing properties in animals, especially using self-administration models.[1,8,10,15,16,22,24] Various possibilities have been put forward to explain such a failure. One of these is that Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive constituent of cannabis, is ineffective in sustaining self-administration because of the relatively delayed onset and lengthy duration of effects. Other possible explanations are that Δ^9-THC has a low abuse liability or that aversive effects could be predominant, thus masking the appetitive properties and accounting for its failure to serve as positive reinforcer in the self-administration paradigm. Over the last few years the discovery of specific receptors for cannabinoids[18,20] has led to the synthesis of specific, potent agonists and antagonists for these receptors. The latter compounds constitute valuable tools in the investigation of the neurobiology of the endogenous cannabinoid system. In an attempt to verify whether specific stimulation of the brain cannabinoid receptor (CB_1)[18] could establish self-administration, we used the CB_1 agonist WIN 55,212-2[2] in drug-naive mice. As previously described[11–14,17] mice were tested in pairs in identical test cages. Each test cage presented a frontal hole provided with an infra-red detector that activated a cumulative recorder and operated a syringe pump to deliver solution contingent on a nose-poke response (NPR). A rear vertical chink was made on the opposite wall through which the tail was extended outside the box and secured to a horizontal surface allowing access to the lateral tail veins with a 27 g winged needle, connected to a syringe through Teflon tubing. Each nose-poke of the active mouse resulted in a contingent injection of 1.0 µl of either vehicle or the drug dissolved in the vehicle both to the active and yoked passive mouse. Nose-pokes of the yoked controls were counted but had no programmed consequences. Mice were first placed in the test cage for 10 min (pretest) during which the tail was taped but no needle was inserted. Pairs of animals were selected on the basis of approximately equal levels of nose-poking during pretest and randomly allocated to the different experimental groups. Each mouse was used in only one self-administration session.

As shown in Table 1, no statistically significant difference was observed in the mean number of NPRs between active and passive mice when vehicle

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Abbreviations: CB_1, brain cannabinoid receptor-1; NPR, nose-poke response; R, reward index; Δ^9-THC, Δ^9-tetrahydrocannabinol.

Table 1. WIN 55,212-2 i.v. self-administration in drug-naive mice

WIN 55,212-2 mg/kg per injection	A	P	R
0 (Vehicle) [27]	20.9 ± 1.47	23.7 ± 2.13	0.9
0.01 [32]	24.2 ± 2.79	24.3 ± 3.76	1.0
0.05 [34]	32.1 ± 3.11**	19.9 ± 2.28	1.6
0.1 [21]	38.8 ± 7.82**	17.4 ± 1.92	2.2
0.5 [18]	12.9 ± 1.81*	25.9 ± 4.35	0.5

Male CD_1 mice (Harlan Nossan, Italy) weighing 25–28 g were used. On arrival, animals were housed six per cage and acclimatized to laboratory conditions (12 h light:12 h dark cycle, lights on at 08:00, 21 ± 1°C room temperature, 60% relative humidity) for at least one week prior to use. Food and water were available *ad libitum* until the time of testing. R (+) WIN 55,212-2 mesylate (RBI, MA, U.S.A.) was freshly dissolved in heparinized (1%) saline-cremophor solution (9:1) at the beginning of the experiments. In order to obtain gradual measurements of the reinforcing effect of the drug solution the ratio, or reward index, (R) between the number of nose-pokes of the active and the yoked passive mouse during a 30-min period of self-administration was used.[14] Therefore the effect was considered rewarding, neutral or aversive when R was greater than, equal to, or less than 1, respectively. A = mean ± S.E. of nose-pokes of the active mice for each group. P = mean ± S.E. of nose-pokes of the passive mice for each group. Number of pairs are indicated in square parentheses. Significance was determined by means of one-way analysis of variance followed by Newman–Keuls test.

$F_{9,254}$=2.51, P<0.01; *P<0.05 and **P<0.01 (vs vehicle and corresponding P).

Table 2. SR 141716 A i.v. self-administration in drug-naive mice

SR 141716A mg/kg per injection	A	P	R
0 (Vehicle) [17]	18.4 ± 2.16	18.3 ± 1.19	1.0
0.05 [18]	21.7 ± 5.98	17.5 ± 1.19	1.2
0.1 [18]	18.9 ± 1.20	21.2 ± 1.98	0.9
0.5 [18]	23.4 ± 2.67	26.2 ± 3.01	0.9

Animals and methodological details as in Table 1. SR141716 A (Sanofi, Montpellier, France) was freshly dissolved in heparinized (1%) saline-Tween 80 (1%) solution at the beginning of the experiments. A, P and R as described in Table 1 footnote. Number of pairs are indicated in square parentheses.

$F_{3,134}$=2.96, not significant.

injections were contingent to NPRs. Therefore, under these conditions the reward index (R) did not differ from 1. Increasing concentrations of WIN 55,212-2 significantly influenced self-administration in active mice, while no differences were observed in NPRs of passive yoked mice with respect to vehicle. WIN 55,212-2 influenced R in a concentration-dependent manner. Thus, the lowest concentration tested, 0.01 mg/kg per injection, failed to significantly modify R. Concentrations of 0.05 and 0.1 mg/kg per injection significantly increased R and were therefore considered to possess reinforcing properties. The highest WIN 55,212-2 concentration tested (0.5 mg/kg per injection) induced a significant decrease in NPRs of active mice and therefore R was lower than 1, indicating an aversive effect for such a dose. Table 2 shows that when the cannabinoid receptor antagonist SR 141716A[21] was made available contingent to NPRs at varying concentrations (0.05–0.1–0.5 mg/kg per injection), no significant differences were observed between NPRs of active and passive yoked mice. However, as shown in Table 3 pretreatment with SR141716A at a dose of 0.25 mg/kg, i.p., completely antagonizes WIN 55,212-2 self-administration.

Our results indicate that the synthetic cannabinoid receptor agonist WIN 55,212-2 is intravenously self-administered by drug-naive mice according to a concentration-dependent bell-shaped curve. Thus, under our experimental conditions the highest positive reinforcing effect was obtained at a concentration of 0.1 mg/kg per injection, while the concentration of 0.5 mg/kg per injection induced aversion, showing that WIN 55,212-2 is able to induce both positive and negative reinforcing effects, depending on the concentration used. Such a response to WIN 55,212-2 is not qualitatively dissimilar to responses obtained in this particular model of self-administration with "classical" drugs of abuse such as cocaine, morphine, nicotine.[14,17] Our results differ from other data present in the literature[1,8,10,15,16,22,24] which fail to demonstrate reinforcing effects of cannabinoids, in particular Δ^9-THC. Possible explanations might be afforded by the use of a different experimental model, different animal species or different pharmacokinetic properties between WIN 55,212-2 and other cannabinoids. The specific cannabinoid receptor antagonist SR 141716A did not induce either positive or negative reinforcing effects, however a small dose of SR 141716A was able to completely antagonize the WIN 55,212-2 induced self-administration in mice. The latter results indicate that specific stimulation of cannabinoid receptors induces self-administration in mice. The neurochemical mechanisms responsible for cannabinoid reinforcing effects are not completely clear and are currently subject to further research. Recent studies[23] suggest that stimulation of central cannabinoid receptors leads to an increased dopaminergic activity, as measured by *in vivo* microdialysis, in mesolimbic areas, particularly in the shell of the nucleus accumbens, in a fashion similar to drugs of abuse such as heroin, cocaine and nicotine. Electrophysiological studies have recently shown an enhanced firing rate of mesolimbic dopaminergic neurons in rats treated with cannabinoid agonists.[6,7] Furthermore it has been proposed that cannabinoid-induced increase of dopamine release is regulated by an endogenous opioid system, due to the fact that the latter effect was prevented by opioid receptor antagonists naloxone and naloxonazine.[23] Whether or not the

Table 3. Antagonism of SR 141716 A on WIN 55212-2 i.v. self-administration in drug-naive mice

Pretreatment	Self-administration	A	P	R
Vehicle a [8]	Vehicle b	16.4 ± 1.16	16.9 ± 1.70	1.0
SR141716A [8]	Vehicle b	19.4 ± 2.80	17.8 ± 3.40	1.1
Vehicle a [8]	WIN 55,212-2	36.4 ± 4.30*	15.9 ± 1.39	2.3
SR 141716A [12]	WIN 55,212-2	17.6 ± 1.78	16.8 ± 1.59	1.0

Animals and methodological details as in Tables 1 and 2. SR 141716 A was freshly dissolved in saline-Tween 80 (1%) solution (Vehicle a) and injected i.p. 30 min before the self-administration session at a dose of 0.25 mg/kg in a volume of 300 µl/mouse. WIN 55,212-2 was freshly dissolved as described in Table 1 footnote (Vehicle b) at a concentration of 2.6 mg/ml corresponding to 0.1 mg/kg per injection. A, P and R as described in Table 1 footnote. Number of pairs are indicated in square parentheses.
$F_{2,14}$=3.12, P<0.01; *P<0.01 (vs all groups and corresponding P).

increased dopamine release can account for reward and abuse liability of cannabinoids is still a matter of debate.[3–5,9,19,23,25] appropriate circumstances, animals can also be rewarded by cannabinoids.

CONCLUSION

Our results strongly suggest that reward induced by stimulation of central cannabinoid receptors is not an exclusive human prerogative but that, under

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